SHORT COMMUNICATION

Occurrence of pathogens in the river–groundwater interface in a losing river stretch (Besòs River Delta, Spain)

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Abstract

The aim of this study is to investigate the occurrence of faecal indicator and microbial pathogens (bacteria and virus) in the shallow urban aquifer of the Besòs River Delta (NE Spain). To this end, human adenovirus (HAdV) and Norovirus of genogroups I and II (NoV GI and NoV GII) as well as the faecal indicator bacteria (FIB) *Escherichia coli* (EC) and faecal *enterococci* (FE) were monitored in groundwater and in the River Besòs in December 2013 and in July 2104. None of the targeted pathogens were detected in groundwater in December 2013 but contamination of human origin was observed in approximately 50% of the points sampled in July 2014 reaching concentrations up to 99 GC/100 mL for HAdV. Generally, microbial concentrations in river water were higher than those detected in groundwater. This observation indicates that pathogens are naturally attenuated when river water infiltrates and flows through the aquifer, however HAdV were detected at a sampling point located at 380 m from the river in the absence of FIB. The presence of human viral contamination may represent a risk for the use of groundwater as a drinking water source. Further research is needed to understand the dynamics of pathogens in river–groundwater interface over long time periods and a wide range of flow conditions (wet and dry periods) since the urban groundwater of this aquifer might be a valuable drinking water resource in Barcelona especially during drought periods. The methodology followed in this research can be applied to other urban aquifers with similar purposes since the scarcity and contamination of freshwater resources are worldwide issues.

Keywords: Human adenovirus; Norovirus; faecal indicator bacteria; urban groundwater; water resource; reclaimed water

1. Introduction

Often urban areas must pump water resources to cover various aspects of the growing urban water demand or as a strategic resource to cover demand at specific times (Howard and Israfilov, 2002; Vázquez-Suñé et al, 2005; Jurado et al, 2017). In fact, groundwater is used for water supply purposes in many European countries (Six et al., 2015; Smith et al., 2015), however, this is not the case of Barcelona (northeast Spain), where near 70% of water supply (northeast Spain) comes from surface water. Drought periods are relative common in this region (e.g., March et al., 2013) and will increment their frequency in the near future as predict by climate change models (García-Ruiz et al. 2011). Therefore it is necessary to seek for alternative water resources. These considerations lead one to wonder whether urban groundwater can be safely used, including its potential use as drinking water because urban aquifers usually contain a wide range of pollutants including microbial pathogens (Hynds et al., 2014).

Pathogenic microorganisms are infectious agents (i.e., virus, bacteria or protozoa) that can produce many diseases (Craun et al., 2010; La Rosa et al., 2012). Diseases like diarrhoea, gastroenteritis, keratoconjunctivitis, respiratory infections and hepatitis are associated with viruses excreted by humans and often found in environmental samples like groundwater, surface water, storage water and food (La Rosa et al., 2012). For instance, human adenoviruses (HAdV) are responsible for enteric illnesses and respiratory and eye infections and noroviruses (NoV) are recognized to be the major cause viral gastroenteritis (Craun et al., 2010, Jiang, 2006). Pathogens reach urban aquifers through different sources such as water leakage from sewer and septic systems (Gotkowitz et al., 2016), direct well contamination from the surface through poorly constructed and managed wells, urban runoff (Ellis, 2004) and infiltration from contaminated rivers since conventional wastewater treatment does not completely remove and/or inactive viruses (Rusiñol et al., 2015). Once in the aquifer, the fate of pathogens depends on their transport and persistence in groundwater that are controlled by climate (e.g., temperature, rainfall, recharge, etc), the aquifer hydraulic properties (e.g., hydraulic conductivity, porosity, etc.) and the type of pathogen (Bitton and Harvey, 1992). Maximizing the residence times in the subsurface might promote the attenuation of bacteria and viruses from water. The processes that major contribute to the removal of viruses during soil passage are adsorption to mineral particles, inactivation and/or natural degradation (Schijven and Hassanizadeh, 2000).

Viruses have been detected in many groundwater supply systems causing recent waterborne outbreaks worldwide (Beer et al., 2015; Giammanco et al., 2014; Kauppinen et al., 2018). Thus, it is necessary to investigate their occurrence in areas where groundwater can be used as a potential drinking water source or for irrigation purposes. This is the case of the shallow aquifer (about 20 m depth) of the Besòs River Delta (NE Spain, Fig. 1). A recent study concluded that the volume of pumped groundwater to prevent seepage problems in an underground parking lot would be sufficient to supply the whole city of Sant Adrià del Besòs (ca. 37000 inhabitants) but, so far, most of this valuable resource is directly poured into the sewage system (Jurado et al., 2017). The City Council of Sant Adrià del Besòs is interested in developing solutions for the management of water resources and the water cycle in the Besòs Litoral area taking into account groundwater. Hence, there is the urgent need to evaluate groundwater quality. Up to date, many studies carried out in this aquifer reported the presence of contaminants of emerging concern such as pharmaceuticals , personal care products and illicit drugs (Jurado et al., 2012, López–Serna et al., 2013, Serra–Roig et al., 2016) but pathogens such as human viruses have never been investigated.

The monitoring of water quality is based on the detection of faecal indicator bacteria (FIB). However, it has been documented that there is no correlation between the absence of FIB and the presence of viral waterborne pathogens (Girones et al., 2010; Rodriguez–Manzano et al., 2012). Thus, using water quality criteria based on FIB might overcome risks associated to the presence of waterborne viral pathogens (Girones et al, 2010). Therefore, surveillance of indicator viruses such as Human Adenoviruses (HAdV) or specific pathogens would be helpful identifying potential sources of human infection (Bofill–Mas et al., 2000; Bofill–Mas et al., 2006; Carter, 2005; Puig et al., 1994).

This study aims to: (1) investigate the presence of pathogenic HAdV (a virus useful as viral faecal indicator) and NoV and the FIB *Escherichia coli* (EC) and faecal *enterococci* (FE) and (2) elucidate the possible sources of contamination in the shallow urban aquifer of Besòs River Delta. To this end, groundwater and river samples were collected for the analysis of the targeted pathogens in December 2013 (C1) and in July 2014 (C2). Despite this is a brief communication we have considered important to share the preliminary findings because there are some ongoing projects in the aquifers of Barcelona (URBANWAT and UNBIASED). These preliminary results are relevant in the context of Barcelona urban area but also the occurrence and fate of these pathogens are expected to be similar in other urban aquifers and/or hydrogeological contexts affected by urban-induced anthropogenic activities (e.g., leakage from the sewerage systems, intensive groundwater pumping to prevent the seepage to underground structures, etc.).

2. Materials and methods

2.1 Study area

The study area is located in the lower part of the Besòs River Delta (northeast of Barcelona, Spain, Fig. 1). The aquifers of the Besòs River Delta are formed within Quaternary fluvial sediments that rest discordantly on low permeability materials ranging from Paleozoic (slates) to Pliocene (clays). The major aquifers are the shallow unconfined aquifer formed by sands and gravels and the main aquifer, which is a confined aquifer, made of siliceous and carbonate sands. An aquitard, which is constituted of silts and clays, separates the shallow and the main aquifers and almost no flow occurs between them (Vázquez-Suñé et al, 2016; Velasco et al, 2012).

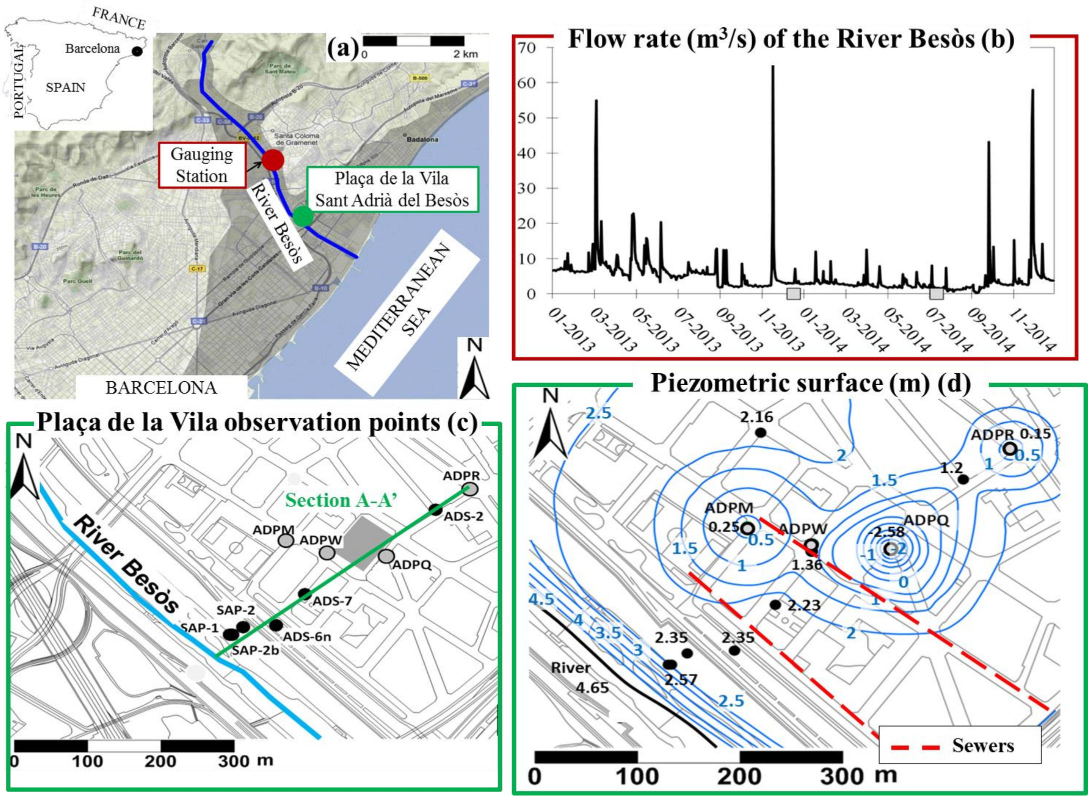

Figure 1. (a) Location of the study area, (b) flow rate of the River Besòs in 2013 and 2014, (c) spatial distribution of the observation points and (d) piezometric surface from the river Besòs to the parking area and sewer pipes (in red dashed line). Note that the piezometric level is in meters above the sea level (m a.s.l.), the grey square symbols in Fig 1b represent the sampling campaigns in December 2013 and in July 2014 and section A–A' is to illustrate the schematic profile in Figure 2. The Catalan Water

Agency (ACA) measures river flow at the Santa Coloma gauging station. (Figure modified from Jurado et al., 2017).

The shallow aquifer is hydraulically connected to the River Besòs at Sant Adrià del Besòs (Vázquez-Suñé et al, 2010; Tubau et al, 2014). The River Besòs stretch in the study area is a losing stream because the groundwater table is below the river water level (Fig. 1d). Hence, the river is the main source of recharge of the aquifer. The climate is typically Mediterranean, with extreme temperatures in January and August, and a yearly average temperature of 15 Cº. Rainfall averages near 600 mm/year and heavy rainfall and flash floods frequently occur. Thus, the River Besòs is characterized to have an irregular flow regime with an average flow of 4.9 m^3/s and reaching up to 65 m^3/s during flood events (autumn 2013, Fig 1b). These events are not constant during the year and vary from one year to another, but they are more common in spring and autumn (e.g., March and October 2013 and December 2014 with river flow above 55 m3/s, Fig. 1b). Conversely, the river flow rate is low in summer. The river flow is measured by Catalan Water Agency (ACA, 2016) at the Santa Coloma gauging station (Fig. 1a).

The residence time of groundwater is about 40 days from the river to the Plaça de la Vila because of the uninterrupted pumping of 150 to 200 L/s to avoid seepage problems in the parking lot (Fig. 1c and 1d) (de Buen, 2009; Ondiviela et al, 2002). The chemical composition of groundwater depends on the seasonal changes in river water quality. Three different river end–members (i.e., recharge sources) were necessary to characterize the temporal variability of the River Besòs: one from the wet season (W1, related to short but intense rainfalls) and two to the dry season (D1 and D2, represent the null or low rainfalls occurring the rest of the year) (Tubau et al., 2014). Among these river end–members, D2 is the major contributor to the resident water of the aquifer in both campaigns (53.2% and 52.4% for C1 and C2, respectively), followed by W1 (44.3% and 44.9% for C1 and C2, respectively) and D1 (2.5% and 2.8% for C1 and C2, respectively) (Jurado et al., 2017). Groundwater is of better quality after the short but intense rain events (i.e., the wet end–member has a high contribution to the total resident water of the aquifer) as the concentrations of most of tracers such as chloride, sulphate and organic micropollutants are diluted in the River Besòs water (e.g., Jurado et al., 2017; Serra–Roig et al., 2016). This dilution effect might also affect the occurrence of pathogens in the aquifer.

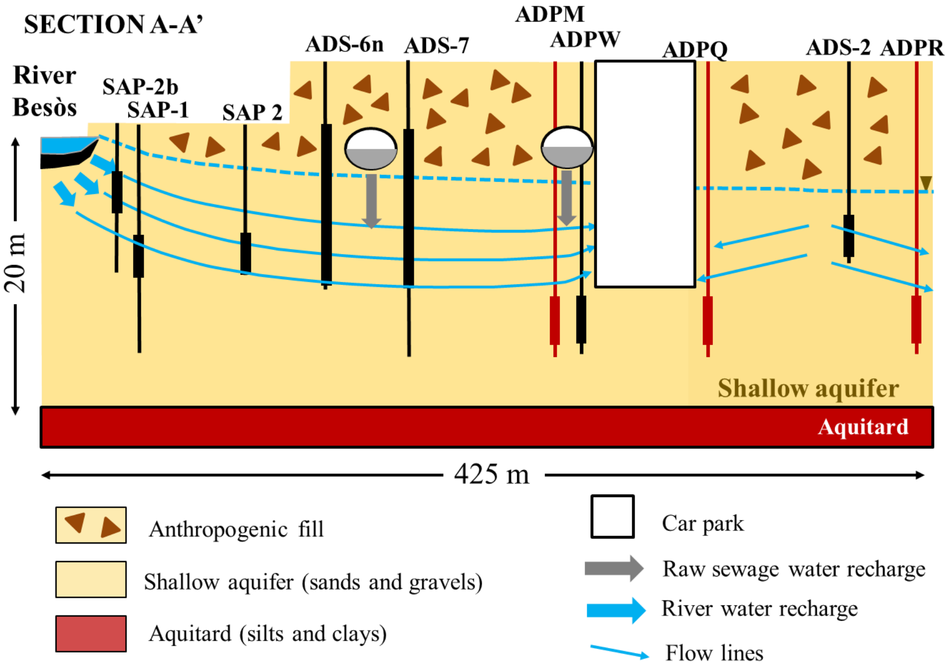

Figure 2. Schematic description of the hydrogeological conceptual model and possible sources of virus contamination in the shallow aquifer: River Besòs (blue arrow) and leaking of raw sewage water (grey arrow). The screen depths of the groundwater observations points sampled are displayed in black and the pumping wells not sampled (ADPM, ADPQ and ADPR) in red.

2.2 Sampling and analytical methods

Two field campaigns were conducted in December 2013 (C1) and in July 2014 (C2) for the analysis of virus of faecal origin, specifically HAdV and NoV of genogroups I and II (NoV GI and NoV GII). The presence of EC and FE was also analysed. Twelve samples were collected from groundwater (SAP–1, SAP–2, SAP–2b, ADS–6n, ADS–7, ADPW and ADS–2, Fig. 1c and Fig. 2), and two from the River Besòs. Groundwater sampling was conducted by pumping while monitoring the field parameters such as dissolved electrical conductivity, pH, temperature and dissolved oxygen (DO). Groundwater samples were collected after pumping a volume of water equal to at least three times the borehole volume and when field parameters were stabilized. Electrical conductivity was measured using a Hanna Groline HI98318 probe with resolution 0.01 mS/cm. Temperature and pH were measured using a waterproof tester Hanna Combo HI98121 with accuracies of 0.1°C for temperature and a resolution of 0.01 for pH. DO was measured using the HI 76407/4 DO probe with a resolution of 0.1 mg/L and an accuracy of 1.5%.

At each sampling point, ten litres of water were collected and analysed in duplicate using the skimmed milk flocculation (SMF) protocol developed in previous studies (Calgua et al., 2013; Gonzales–Gustavson et al., 2017). All samples were spiked with MS2 bacteriophage as a process control. Viral Nucleic acids (DNA and RNA) were extracted from all samples using QIAamp(R) viral RNA Mini Kit (Qiagen, Inc., Valencia, CA) and specific real–time PCRs assays were used to quantify each of the studied viruses including MS2 (Bofill-Mas et al., 2006; da Silva et al., 2007; Hernroth et al., 2002; Kageyama et al., 2003; Loisy et al., 2005; Svraka et al., 2007). The bacterial parameters were quantified using 100 mL of the initial sample. The enumeration of EC was carried out in a 96–well microplate (MUG/EC 355–3782, BioRad, Barcelona, Spain®) according to ISO 9308–2:2012 and FE were quantified in a 96–well microplate (MUG/EC 355–3783, BioRad®) following the ISO 7899–1:1998 procedure.

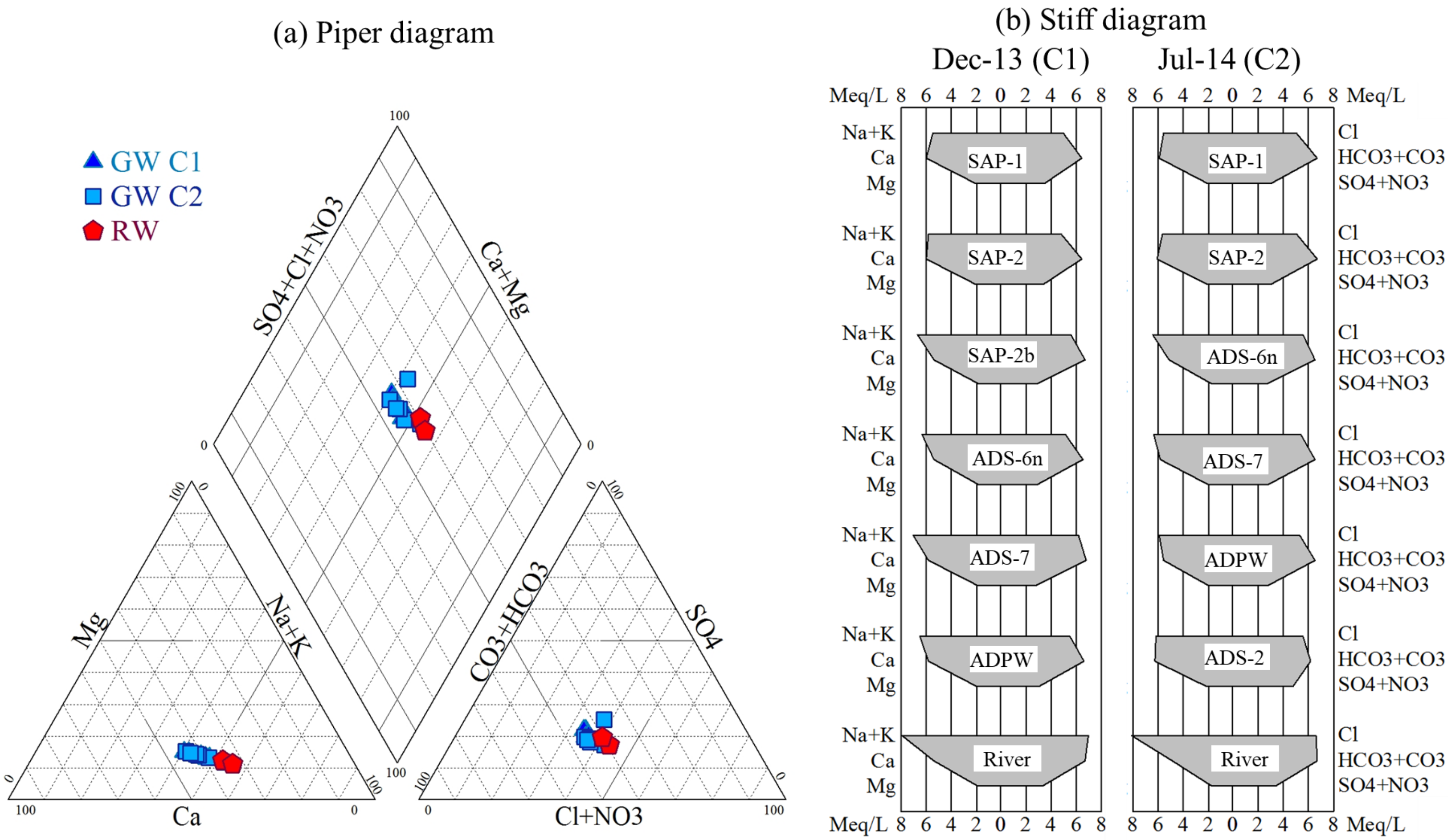

Figure 3. (a) Piper diagram and (b) Stiff diagram showing major ion chemistry of the groundwater (GW) and the river water (RW) in December 2013 (C1) and in July 2014 (C2).

3. Results and discussion

3.1 Hydrochemistry and microorganisms in the River Besòs and groundwater

3.1.1 General hydrochemistry

Electrical conductivity and pH values are slightly higher in river water (average values were 1609±7.1 µS/cm and 7.85±0.07, respectively) than in groundwater (average values were 1397±68.2 µS/cm and 7.15±0.09) and they did not varied in the winter and summer sampling campaigns (Table 1). The river water temperature variation turns out to be large, being 11.5°C in December 2013 (C1) and 25.6°C in July (C2). In contrast, the temperatures of groundwater were more constant with values around 20°C in both sampling campaigns (Table 1). River water presented high concentrations of dissolved oxygen (average was 7.5±0.6 mg/L) and groundwater almost null levels in most observation points (average was 0.35±0.5 mg/L).

Major ion compositions showed that River Besòs and the groundwater in the shallow aquifer presented similar composition, being Cl–(SO_4)–Na–(K) and Cl–(SO_4)–Ca–(Mg) types, respectively (see Piper diagram in Fig. 3a, Simler, 2009). As river water is the main recharge source of the aquifer, most of the major ions showed similar average concentrations in both water compartments (see Stiff diagram in Fig. 3b; 150.5±12.3 vs. 147.5±19.2 mg/L for sulphate, 415±1.1 vs. 403±9.3 mg/L for bicarbonate, 102±7.5 vs. 115.1±6.7 mg/L for calcium and 21.2±1.7 vs. 23.8±1.5 mg/L for magnesium). Somewhat higher average concentrations were found for chloride (242±8.5 vs. 191.4±13.7 mg/L), sodium (170.8±3.8 vs. 133.5±10.3 mg/L) and potassium (20.7±0.2 vs. 13.3±2 mg/L) (Fig. 4). In contrast, average nitrate concentration in river water was the double than that of groundwater (18.5±12.1 vs. 9.1±11.8 mg/L). Moreover, some redox indicators such as DO and total organic carbon (TOC) were much lower in groundwater than in the river (black squares, Fig. 4). These observations might indicate the occurrence of redox processes (i.e., aerobic respiration and denitrification) when river water infiltrated the aquifer resulting in a reducing groundwater environment (evidenced by the null or low concentrations of DO and the presence of ammonium in the aquifer, Table 1 and Fig. 4).

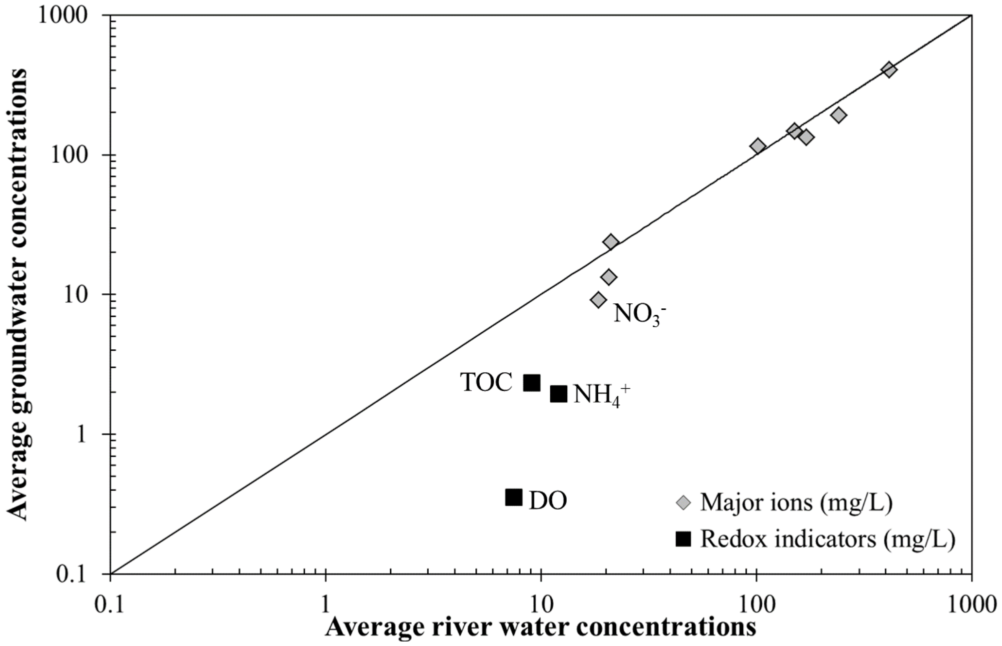

Figure 4. Average concentrations in the River Besòs and in the aquifer for major ions (grey rhombus) and some redox indicators (black squares).

3.1.2 Occurrence of microorganisms in river and groundwater

The concentrations of HAdV, NoV GI and NoV GII and FIB for each sampling campaign are summarized in Table 1. Results confirm the presence of human viruses and bacteria in the river and groundwater samples. HAdV were detected in river samples in both sampling campaigns whilst NoV GI and NoV GII were only detected in the first sampling campaign (C1, December 2013). The concentration of HAdV was of 192 and 118 GC/100 mL at summer and winter campaigns, respectively (Table 1).

Concerning groundwater samples, no viruses were detected in any of the groundwater observation points in December 2013 (C1, Table 1a) while HAdV were detected in half of the samples (SAP–1, ADPW and ADS–2) in July 2014 (C2, Table 1b). The concentrations of HAdV ranged from 14 to 99 GC/100 mL. Only positive groundwater samples for HAdV were tested for NoV GI and NoV GII presence in C2 and none of these tests showed any positive result (Table 1b). The limit of detection (LOD) of the applied methodology for HAdV was of <29.3 GC/100 mL (95% confident interval of 20.2–59.2 GC/100 mL). MS2 concentrations showed that samples were correctly processed for their analysis.

As expected, FIB were more frequently detected in river water than in groundwater samples (Table 1). Levels of both bacteria were much higher in December 2013 than in July 2014 in river water samples, being 8775 vs. 2221 CFU/100 mL for EC and 27335 vs. 403 CFU/100 mL for FE. FIB were only detected in two groundwater samples in July 2014 (SAP–1 and ADS–6n, Table 1b). Remarkably, FE was detected in groundwater observation point SAP–1 in similar concentrations than in the river (449 vs. 403 CFU/100 mL). The LOD of the applied methodology for EC was of <15GC/100 mL.

Spanish regulations for drinking water (RD 140/2003) and different uses of reclaimed water (RD 1620/2007) determined that river water and the observation points SAP–1 and ADS–6n exceeded the threshold of 0 CFU/100 mL of EC and FE set by the RD 140/2003 for drinking water (Table 1b). Reclaimed water uses allowed are: urban (e.g., irrigation of private gardens and street cleaning), agricultural (crop irrigation), industrial, recreational (e.g., golf course irrigation) and environmental (e.g., aquifer recharge and irrigation of green areas). Overall, groundwater fitted better the quality requirements for EC than river water for all the uses. Only EC concentration in SAP–1 exceeded the threshold of 0 CFU/100 mL set by RD 1620/2007 for direct aquifer recharge injection and irrigation of private gardens.

(a)Water samples	**Pathogens**					**Physico–chemical parameters**			**Wastewater indicators**	
	HAdV	NoV GI	NoV GII	EC	FE	Electrical conductivity	DO	T	Nitrate	Ammonium
	GC/100 mL			CFU/100 mL		µS/cm	mg/L	ºC	mg/L	
River	118	3.2	191	8775	27335	1604	7.9	11.5	27.1	14.3
SAP–1	ND	ND	ND	ND	ND	1324	0.11	19.6	12.3	1.2
SAP–2	ND	ND	ND	ND	ND	1321	0.15	20	12.4	1.5
SAP–2b	ND	ND	ND	ND	ND	1329	0.11	19.2	3.2	5.8
ADS–6n	ND	ND	ND	ND	ND	1293	0.1	20.4	4.5	3.3
ADS–7	ND	ND	ND	ND	ND	1460	0.13	21.4	3	2.5
ADPW	ND	ND	ND	ND	ND	1434	1.2	19	0	1.9

(b)Water samples	**Pathogens**					**Physico–chemical parameters**			**Wastewater indicators**	
	HAdV	NoV GI	NoV GII	EC	FE	Electrical conductivity	DO	T	Nitrate	Ammonium
	GC/100 mL			CFU/100 mL		µS/cm	mg/L	ºC	mg/L	
River	192	ND	ND	2221	403	1614	7.1	25.6	10	9.9
SAP–1	14*	ND	ND	46	449	1394	0.15	19.8	13.2	0.75
SAP–2	ND	NA	NA	ND	ND	1389	0.15	20.4	11.6	0.57
ADS–6n	ND	NA	NA	ND	46	1439	0.17	20.6	0	3
ADS–7	ND	NA	NA	ND	ND	1470	0.19	18.9	5.1	1.2
ADPW	99	ND	ND	ND	ND	1400	0.19	19.9	1.4	1.2
ADS–2	60.5	ND	ND	ND	ND	1507	1.6	19.5	43.1	0.5

*Table 1. Concentrations of pathogens, physicochemical parameters and wastewater indicators in river and groundwater in (a) December 2013 (C1) and (b) July 2014 (C2). GC: Genomic Copies. CFU: colony–forming units. ND: Not detected (<29.3 GC/100 mL, in a confidence interval of 95% from 20.2 to 59.2 GC/100 mL for HAdV and <15 UFC/100 mL for bacteria). NA: Not analyzed. * Positive in one sample.*

3.2 Identification of the possible sources of pathogens in the aquifer

As mentioned before, River Besòs is the main water recharge source of the aquifer and thus, the major source of contamination of pathogens. The concentrations of virus and bacteria in river water were 1 or 2 orders of magnitude higher than those found in groundwater in both sampling campaigns (Table 1). These results suggest that they are naturally removed during the river water passage through the aquifer material as previously demonstrated by other authors in riverbank filtration systems (Freitas et al., 2017; Sprenger et al., 2014). For instance, the bank filtration system of River Beberibe (Brazil) showed potential for reduction of EC since it concentration in the river ranged from 280 to ≥160,000 NMP per 100 mL but were absent in a production well located 15 meters from the river (Freitas et al., 2017). Similarly, viruses were significantly removed from the highly contaminated River Yamuna (in central Delhi, India) by a factor of 10^4 and 10^6 at 4 m and 50 m filtration distance, respectively (Sprenger et al., 2014). The concentrations of HAdV and NoV in river water were 3.6×10^4 and 5.4×10^4 GC/100 mL and none of them were detected in the observation well located at 50 m. Enteric viruses due to their small size might travel further distance than bacteria and they can survive longer periods of time (Betancourt et al., 2014). In fact, viruses were found at long distance from the river in the absence of FIB in the shallow aquifer of the Besòs River Delta (Table 1). HAdV were detected in July 2014 (C2) in two observation points that are located in the surroundings of the Plaça de la Vila underground parking lot (ADPW and ADS–2, Table 1b). This fact could be related to some rain events occurred before the second sampling campaign (July 2014, C2) that increased the river flow rate (Fig 1b). As pointed out by Derx et al. (2013), the fluctuations in river water level cause viruses to be transported at higher concentrations into the riverbank. These authors postulated that increasing the water level between 1 and 5 m caused in increasing virus concentrations with 2–4–log and decreasing the travel time with 30%. However, HAdV were not detected along the groundwater flow path from (SAP–1, SAP–2, ADS–6n and ADS–7, Fig. 2). This observation might suggest: (1) a high concentration of viruses in the river that were later diluted with the river flow increase and/or (2) an additional source of virus contamination such as leakage from the sewage system. The second hypothesis seems to be realistic since a previous study quantified that loss from sewage system contributed 8% (in the observation point ADPW) and 16% (in the observation point ADS–2) to the resident groundwater (Jurado et al., 2013) (Fig. 2). In addition, for supporting this hypothesis, the occurrence of HAdV in these points was compared with some of the main wastewater indicators in urban areas such as nitrate (Wakida and Lerner, 2005). Nitrate concentration and electrical conductivity in ADS–2 displayed the highest values among all groundwater samples in July 2014 (C2), being 43.1 mg/L and 1507 µS/cm (Table 1b, Fig. 5), which would indicate that an additional water source contributed to the aquifer recharge. In contrast, ADPW had a low concentration of nitrate (1.42 mg/L), as denitrification could have occurred along the groundwater flow path (blue arrow, Fig. 5), but HAdV were also detected. The occurrence of such process in both sampling campaigns is supported by the progressive decrease of nitrate concentrations from the groundwater samples collected near the river (SAP–1 and SAP–2 with nitrate concentrations above 10 mg/L) to ADPW (Table 1, Fig. 5 for C2) and the reducing conditions of the aquifer (with average DO concentration of 0.2 mg/L and the presence of ammonium, Table 1). In that case, the analysis of stable isotopes, such as those boron and nitrate, would help to identify additional recharge sources.

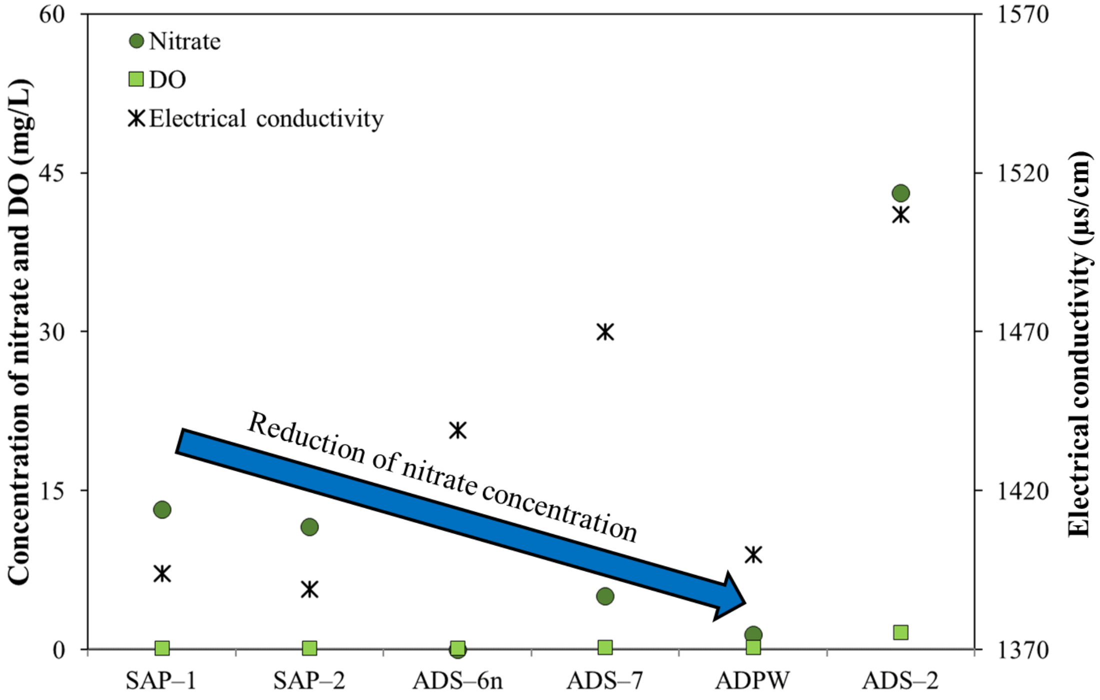

Figure 5. Nitrate and DO concentrations (mg/L, primary axis) and electrical conductivity (µs/cm, secondary axis) for groundwater samples in July 2014 (C2). The blue arrow represents the reduction of nitrate concentration along the flow path.

4. Conclusions and future prospects

This study is the first attempt to investigate the microbial contamination of the shallow urban aquifer of the Besòs River Delta in the city of Sant Adrià del Besòs (NE Spain), where urban groundwater might be used as potential resource of drinking water. Results obtained confirm the prevalence of human viruses and bacteria in the River Besòs in December 2013 and in July 2014. Virus concentrations (HAdV) and their detection frequencies in groundwater were high in July 2014, suggesting seasonal variations in their occurrence in the aquifer. Overall, pathogen concentrations were higher in river than in the aquifer, suggesting that they are naturally attenuated when river water infiltrates the aquifer. Hence, groundwater microbiological parameters (EC and FE) fitted the thresholds set up for drinking water (RD 140/2003) and reclaimed water uses (RD 1620/2007).

HAdV were detected at two sampling points (ADPW and ADS–2) located at more than 200 m from the river in the absence of FIB. The presence of HAdV in these points might indicate the long stability of HAdV in groundwater filtered from the river or the possibility of additional sources of groundwater contamination such as loss from sewer network. Further research is needed to elucidate this observation. For example, the use of stable isotopes (i.e., nitrate and boron) would help to identify the different recharge sources in future sampling campaigns.

Given the limited number of collected water samples, we suggest that future research efforts should be focused on investigating the dynamics of pathogens in river–groundwater interface over long periods of time (e.g., hydrological year) and different flow conditions (prevalence of wet and dry periods). The occurrence of viruses in groundwater is characterized by high temporal variability and, therefore, using them as tracers requires more frequent sampling than other groundwater tracers. This additional research will allow: (1) better constraining the potential sources of contamination, (2) the appropriate management of urban groundwater resources to prevent enteric pathogen contamination that has been associated with disease outbreaks and (3) defining suitable treatments for the safe use of groundwater as an alternative resource for drinking water or other potential uses (e.g., restore the ecological flow of the river in summer, prevent salt–water intrusion, etc.). The methods and results of this research can be useful to other urban aquifers with similar purposes since the availability of freshwater of good quality is a worldwide issue.

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